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Out of East Asia: *Epermenia sinjovi* Gaedike, 1993 (Lepidoptera, Epermeniidae) newly recorded from the European Alps

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Abstract. *Epermenia sinjovi* Gaedike, 1993, a species with a hitherto westernmost known occurrence in Central Siberia, is recorded from the European Alps (Tyrol, Austria and Bavaria, Germany). Its identification is based on DNA barcodes and morphological characteristics. The probability of a recent introduction is considered as very low due to the remoteness and wild nature of the habitat, and it seems likely that the species was previously overlooked due to its similarity to *Epermenia chaerophyllella* (Goeze, 1783).

Keywords. DNA barcode, Eastern Asia, Austria, Germany, overlooked species, *Apiaceae*

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Introduction

The family Epermeniidae comprises roughly 190 species in 12 genera (van Nieukerken et al. 2011; Gaedike and Mally 2014; Sohn and Wu 2015; Gaedike 2022), with probably many still undescribed species. So far, 26 species have been recorded from Europe, 15 of which belong to the genus *Epermenia* Hübner, [1825] (Lepiforum 2022a; Gaedike 2022). Species delimitation based on morphology is usually straightforward and furthermore supported by DNA barcode sequences of the mitochondrial cytochrome oxidase subunit 1 (COI) gene with comparatively low intraspecific and high interspecific divergences, based on a nearly complete DNA barcode library of the European fauna. It, therefore, came as a surprise when independent sequencing of two specimens initially determined as *E. chaerophyllella* (Goeze, 1783) resulted in a distinct DNA barcode cluster and Barcode Index Number (BIN) (Ratnasingham and Hebert 2013). Early tentative efforts to identify the second (rare) cluster failed, and the problem remained untouched for a couple of years. In the context of a recently prepared work on the genus, the problem was re-assessed. In the meantime, Chinese colleagues successfully sequenced the East Asian *E. sinjovi* Gaedike, 1993 and uploaded several full DNA barcodes to the Barcode of Life Data System (BOLD) database (Wang et al. 2021). These DNA barcodes fully correspond with European samples. Therefore, the results finally enabled us to identify our mystery species as the same as from Central Europe. *Epermenia sinjovi*, due to its similarity to the widespread European *E. chaerophyllella*, may cause future taxonomic confusion without DNA barcoding.

Methods

Two male specimens initially determined as *Epermenia chaerophyllella* were collected at UV light and subsequently prepared for DNA barcoding. This method usually allows accurate species identification from a 658-base-pair long segment of the COI gene. Tissue samples were prepared following the prescribed standards (deWaard et al. 2008) and successfully processed at the Canadian Centre for DNA Barcoding (CCDB;

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Biodiversity Institute of Ontario, University of Guelph). In addition, 56 public sequences >570 bp of three *Epermenia* species (five *E. sinjovi*, 32 *E chaerophyllella*, and 19 *E. albapunctella* (Busck, 1908)) were used from BOLD (Ratnasingham and Hebert 2007; Ratnasingham 2018) as a reference for species separation (Table 1). Details including complete voucher data and images of these specimens can be accessed in the public dataset "*Epermenia chaerophyllella* species-group" https://doi.org/10.5883/ds-eperchae and among public data in BOLD. Sequences from the dataset were finally submitted to GenBank.

Degrees of intra- and interspecific variation of DNA barcode fragments were calculated under the Kimura 2-parameter model of nucleotide substitution using analytical tools of BOLD systems v. 4.0. (http://www.boldsystems.org). Calculation of intraspecific distance was furthermore normalized with BOLD calculation tools to reduce bias in sampling at the species level. A neighbor-joining tree of DNA barcode data from the dataset was constructed using MEGA 11 (Tamura et al. 2021) under the Kimura 2-parameter model for nucleotide substitutions.

Photographs of adults were taken with a Zeiss Stemi 508 KMAT stereomicroscope and photographs of genitalia with a Zeiss Axiolab 5 microscope, both adapted to an Olympus OM-D Mark III camera. Stacked photos were edited using Helicon Focus v. 4.8 and Adobe Photoshop v. 6.0.

Results

Epermenia sinjovi Gaedike, 1993

Epermenia sinjovi Gaedike 1993: 99, figs 14–22.

New records. AUSTRIA – Tyrol • Brandenberg, Ellbachtal, unterer Kaiserboden; 47°32′20″N, 011°55′34″E; 780 m alt.; 21.V.2014; P. Huemer leg.; DNA Barcode ID TLMF Lep 16303; 1 ♂, genitalia slide EPE 11 P. Huemer, Tiroler Landesmuseum Ferdinandeum, Innsbruck. GERMANY – Bavaria • Berchtesgaden, Königsbach, Wasserfall; 47°34′41″N, 012°59′53″E; 600 m alt.; 25.VI.2019; A. Haslberger leg.; DNA Barcode ID BC ZSM Lep 109027; 1 ♂, research collection of Alfred Haslberger, Teisendorf.

Identification. The identification of *E. sinjovi* was initially based on DNA barcode reference sequences in BOLD originating from China (Fig. 1) (Wang et al. 2021). *Epermenia sinjovi* and its closest relatives, *E. chaerophyllella* and *E. albapunctella*, cluster in different BINs (Ratnasingham and Hebert 2013). *Epermenia sinjovi* (BIN BOLD:ACS3942) can be easily separated from the nearest neighbor *E. albapunctella* (BIN BOLD:AAF0142) by a *p*-distance of 5.98% and from *E. chaerophyllella* (BIN: BOLD:AAC9729) by 6.49%. Furthermore, adult morphology was studied (Figs. 2, 3) and compared with to the literature. The forewings are extremely variable in colour, with four distinct forms (Kuroko and Gaedike 2006; Wang et al. 2021), but according to Gaedike (1966) the adult of *E. sinjovi* differs

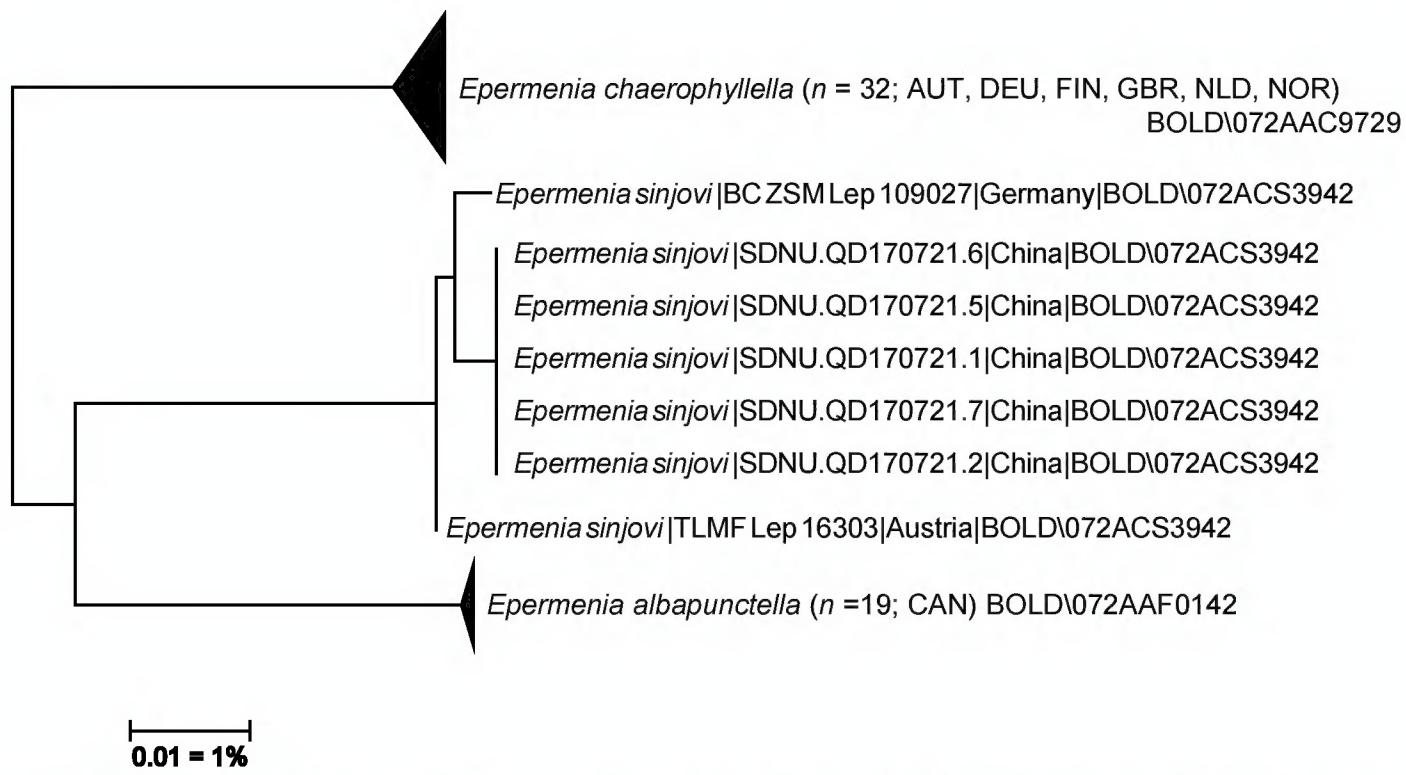

Figure 1. Neighbor-joining tree of *Epermenia* (Kimura 2-parameter, built with MEGA 11 (Tamura et al. 2021); source: DNA Barcode data from BOLD (Ratnasingham 2018).

Table 1. List of sequenced specimens of *Epermenia*.

Identification	Sample ID	Process ID	GenBank	Country
E. albapunctella	BIOUG05594-B07	SMTPB8854-13	KT135965	Canada
E. albapunctella	BIOUG08639-F10	SMTPD3037-13	KT142180	Canada
E. albapunctella	BIOUG08824-H02	CNROK1101-13	KR445714	Canada
E. albapunctella	BIOUG10526-D02	CNGBI1582-14	KR452433	Canada
E. albapunctella	BIOUG10526-D04	CNGBI1584-14	KR454416	Canada
E. albapunctella	BIOUG10771-C09	PHNXN603-14	KT129112	Canada

Identification	Sample ID	Process ID	GenBank	Country
E. albapunctella	BIOUG13073-G05	SMTPF8666-14	KT147787	Canada
E. albapunctella	BIOUG13115-B07	SMTPG171-14	KT130147	Canada
E. albapunctella	BIOUG13129-D03	SMTPG1922-14	KT134050	Canada
E. albapunctella	BIOUG21383-B08	CNTIJ657-15	MG467018	Canada
E. albapunctella	BIOUG21839-A04	CNTIG287-15	MG465338	Canada
E. albapunctella	BIOUG21901-A02	SMTPL089-15	MG362945	Canada
E. albapunctella	BIOUG21930-D07	SMTPL3752-15	MG362528	Canada
E. albapunctella	BIOUG32583-A09	ELPCG3896-17		Canada
E. albapunctella	BIOUG33470-A05	OPPIA348-17		Canada
E. albapunctella	BIOUG33470-A08	OPPIA351-17		Canada
E. albapunctella	BIOUG35041-B06	OPPEQ246-17		Canada
E. albapunctella	AC005141	LQAC026-06	KT125371	Canada
E. albapunctella	BARS_2016_26_222	BARSM169-17	MG364745	Canada
E. chaerophyllella	BC ZSM Lep 23100	FBLMS233-09	HM901996	Germany
E. chaerophyllella	BC ZSM Lep 71009	FGMLB703-13		Germany
E. chaerophyllella	BC ZSM Lep 78398	GBLAB873-13		Germany
E. chaerophyllella	BC ZSM Lep 89242	FGMLG411-15		Germany
E. chaerophyllella	BC ZSM Lep 92990	FGMLG549-16		Germany
E. chaerophyllella	BC_LSNOE_Lep_01487	NOEGE727-19		Austria
E. chaerophyllella	BIOUG15568-A01	GMNWF1893-14		Norway
E. chaerophyllella	BIOUG15568-C08	GMNWF1924-14		Norway
E. chaerophyllella	BIOUG15678-F05	GMNWG3070-14		Norway
E. chaerophyllella	BIOUG16269-D04	GMGMB160-14		Germany
E. chaerophyllella	FG20	GRPAL895-12	KF367703	United Kingdom
E. chaerophyllella	KLM Lep 01321	PHLAJ276-14		Austria
E. chaerophyllella	KLM Lep 03738	PHLAJ603-15		Austria
E. chaerophyllella	KLMLep15977	LEASX1006-23		Austria
E. chaerophyllella	MM00443	LEFIA041-10	HM396390	Finland
E. chaerophyllella	MM09752	LEFIA875-10	HM387013	Finland
E. chaerophyllella	MM09795	LEFIA917-10	HM387053	Finland
E. chaerophyllella	MM14007	LEFIG153-10	HM875833	Finland
E. chaerophyllella	MM25201	LEFIJ4882-16	MZ611325	Finland
E. chaerophyllella	NHMO-DAR-8192	LON2696-16		Norway
E. chaerophyllella	NHMO-DAR-14028	LON6503-17		Norway
E. chaerophyllella	RMNH.INS.546114	LEAFN190-13		Netherlands
E. chaerophyllella	RMNH.INS.552182.1	LEAFN345-13		Netherlands
E. chaerophyllella	RMNH.INS.552182.2	LEAFN346-13		Netherlands
E. chaerophyllella	RMNH.INS.554623.1	LEAFN664-13		Netherlands
E. chaerophyllella	RMNH.INS.554623.2	LEAFN665-13		Netherlands
E. chaerophyllella	TLMF Lep 08211	PHLAH392-12	KM572603	Austria
E. chaerophyllella	TLMF Lep 26728	LEASU892-18		Austria
E. chaerophyllella	UKLB16A01	CGUKB412-09		United Kingdom
E. chaerophyllella	UKLB34E12	CGUKD169-09	KX043325	United Kingdom
E. chaerophyllella	UKLB42H10	CGUKD952-09	KX043567	United Kingdom
E. chaerophyllella	UKLB43D01	CGUKD990-09	KX044017	United Kingdom
E. sinjovi	BC ZSM Lep 109027	NPBLA386-20		Germany
E. sinjovi	TLMF Lep 16303	ABOLA616-14		Austria
E. sinjovi	SDNU.QD170721.1	EPECN001-21		China
E. sinjovi	SDNU.QD170721.2	EPECN002-21		China
E. sinjovi	SDNU.QD170721.5	EPECN003-21		China
E. sinjovi	SDNU.QD170721.6	EPECN004-21		China
E. sinjovi	SDNU.QD170721.7	EPECN005-21		China

Figure 2. *Epermenia sinjovi*, adult male, Austria. Scale bar: 5 mm.

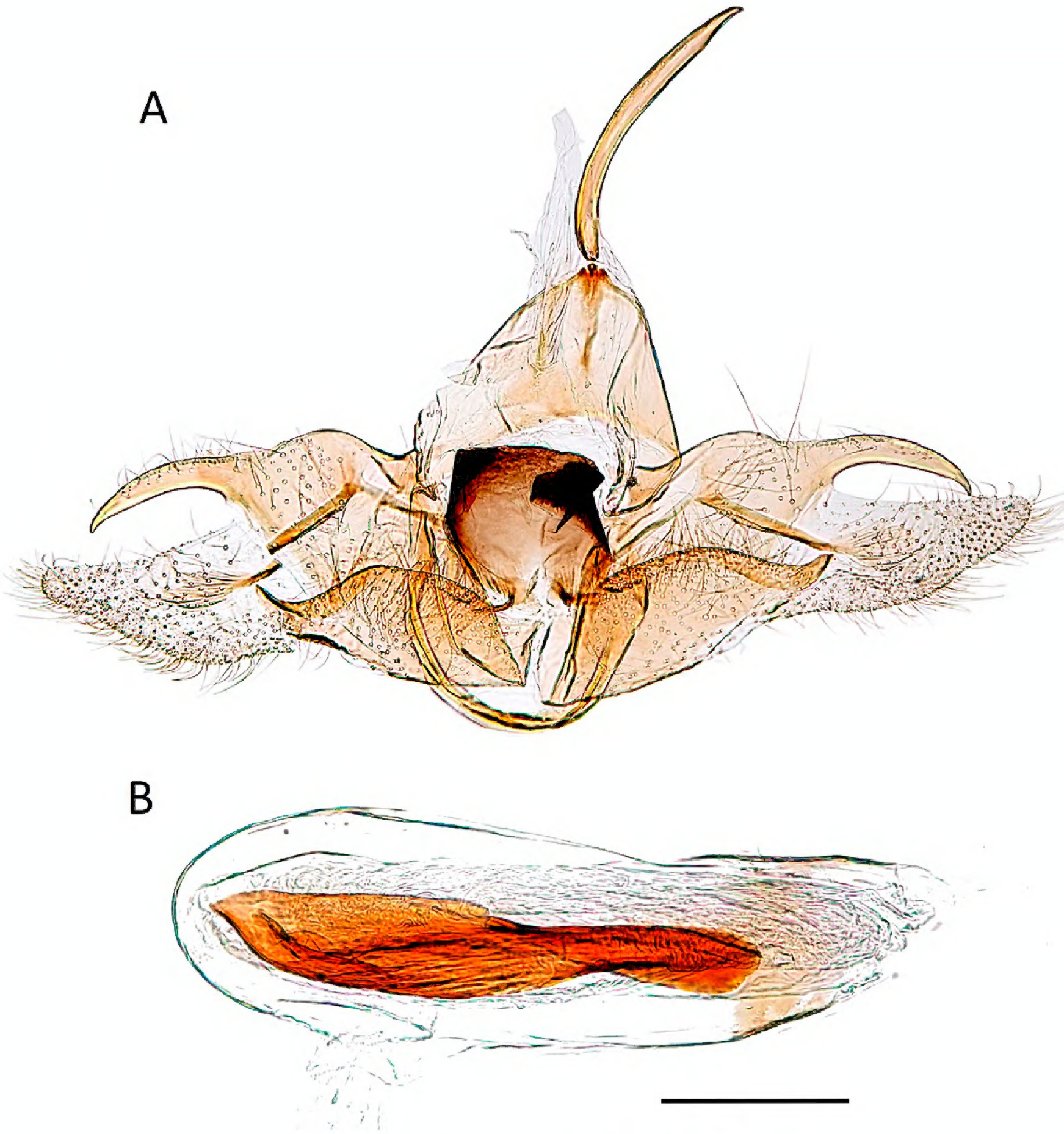

Figure 3. *Epermenia sinjovi*, male genitalia, Austria. **A.** Genitalia capsule. **B.** Phallus. Scale bar: 20 µm.

from *E. chaerophyllella* by three rather than four scale dents along the dorsum. However, this character is difficult to assess in worn specimens. Male genitalia of *E. sinjovi* are characterized by a single, large, though highly variable (Wang et al. 2021) cornutus and a folded vesica of the phallus. In *E. chaerophyllella* a smaller, second cornutus is present and the vesica is inconspicuous (see Gaedike 1966; Lepiforum 2022b). Females could not be examined by us, but extensive descriptions of the genitalia and external morphology have been published by Kuroko and Gaedike (2006) and particularly Wang et al. (2021), who also described the biology in detail, and are not repeated herein

Distribution. The species is widely distributed in Asian Russia, ranging from Kamtchatka and Primorskii region in the east to Krasnoyarsk region in the west (Sinev 2019). It is furthermore known from Japan (Kuroko and Gaedike 2006) and the Shandong province of China (Wang et al. 2021) (Fig. 4).

Biology. The larval ecology in Europe is unknown but probably corresponds to observations published from Eastern Asia. According to Kuroko and Gaedike (2006) this species was bred from *Angelica pubescens* Maxim. and *A. ursina* (Rupr.) Maxim. (Apiaceae) in Japan and from *A. polymorpha* Maxim. in China (Wang et al. 2021), indicating *Angelica* spp. as potential host-plants for European populations. The related *E. chaerophyllella* is reported from *Chaerophyllum* L., *Conium* L., *Pimpinella* L., *Sium* L., *Seseli* L., *Silaum* Mill., *Angelica* L., *Heracleum* L., *Pastinaca* L., *Daucus* L., *Anthriscus* (Pers.) Hoffm., *Apium* L., *Carum* L., *Sison* L., *Torilis* Adans., *Oenanthe* L., *Levisticum* L., and *Peucedanum* L. (Apiaceae) (Budashkin and Gaedike 2005). Young larvae of that species are initially leaf miners but later feed from a slight web on the underside of a leaf (Sterling et al. 2012).

Habitat. The habitat in Tyrol (Austria) is a remote, south-exposed, open beech–spruce forest on limestone, intermixed with areas of grassland and xerophilous rock and scree formations. It is well known for some spectacular accompanying faunal elements, i.e. the famous *Arctia matronula* (Linnaeus, 1758) with one of the largest relict populations in Central Europe. Also, the habitats in Bavaria (Germany) are largely natural with similar structures and vegetation.

Discussion

Epermenia chaerophyllella, a widely distributed European species, which is also known from western Asia (Gaedike 1993), has hitherto been considered an unmistakable species on this continent. It differs from the few externally somewhat similar species in Europe, *E. aequidentellus* (E. Hofmann, 1867) and *E. strictellus* (Wocke, 1867), by the slightly hooked wing apex and the usually blackish-brown forewings. The more closely

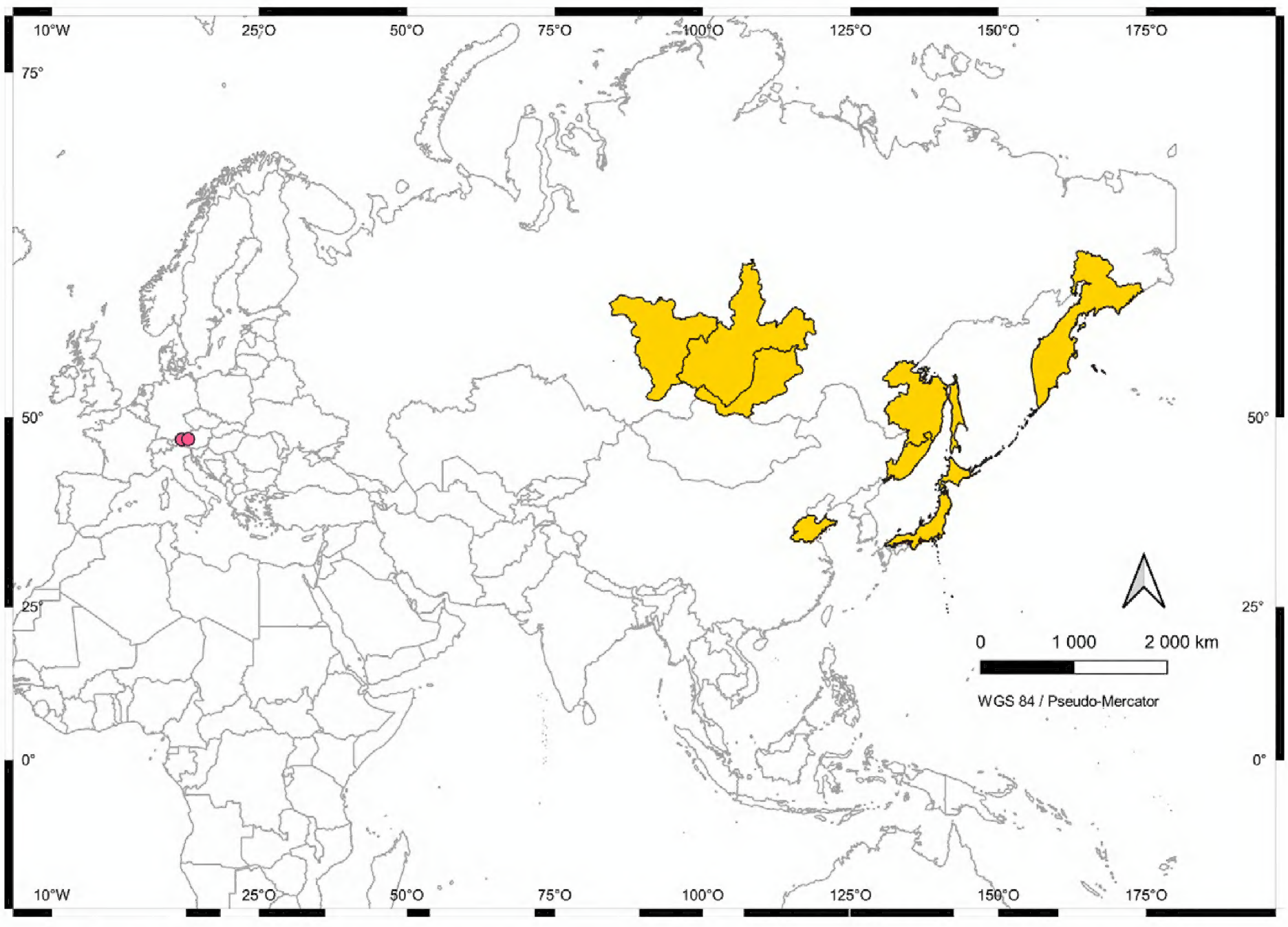

Figure 4. *Epermenia sinjovi*, actual province (Russia and China) and country (Japan) distribution (in yellow) and new records (red dot) (provincial/country data sources: Wang et al. 2021; Kuroko and Gaedike 2006; Sinev 2019) (base map: https://gadm.org/).

related Eastern Asian *E. sinjovi* can be separated by the structures of male and female genitalia (Gaedike 1993; Kuroko and Gaedike 2006). The unexpected discovery of the Eastern *E. sinjovi* approximately 5000 km from the nearest documented population raises some important questions. Is it a widespread Palaearctic species that has so far been overlooked in Europe and western Asia, or is it, rather, an anthropogenic introduction? Especially in the last few decades, an increasing number of East Asian moths, which are most probably the results of recent introductions, have been recorded in Europe. Examples are found in Gracillariidae (Kirichenko et al. 2017; Lopez-Vaamonde et al. 2021), but also in several other families such as Cosmopterigidae (*Cosmopterix feminella* Sivev, 1988; Koster et al. 2019), Scythrididae (*Scythris sinensis* (Felder & Rogenhofer, 1875); Landry et al. 2013), Tortricidae (*Aterpia circumfluxana* (Christoph, 1881); Trematerra and Huemer 1994) and Crambidae (*Cydalima perspectalis* (Walker, 1859); Mally and Nuss 2010). Typically, all of these species are primarily restricted to anthropogenic habitats such as parks, gardens, or seminatural grassland, at least in the initial stage of the new colonization processes. However, the remoteness of the hitherto known sites in the Alps probably indicates a natural occurrence. It can be assumed that *E. sinjovi* was simply overlooked, mainly because of its similarity to *E. chaerophyllella*, a species, which was previously considered unmistakable in Europe. Without the comprehensive barcode screening that has been undertaken in the Alps for a number of years, the species would probably have remained undiscovered. The new discovery furthermore demonstrates the great importance of the numerous DNA barcoding projects all over the world. Without reference sequences from China, *E. sinjovi* would probably have been unidentified for a long time and, in the worst case, even have been described again. It seems likely that the species is more widespread in Europe, and an unverified genital image from northwestern Essex, England (Wheeler et al. 2023) possibly also belongs to *E. sinjovi*.

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Author Contributions

Conceptualization: PH, AH, AHS. Data curation: PH, AH, AHS. Formal analysis: PH, AHS. Funding acquisition: AH. Methodology: PH. Validation: PH, AH, AHS. Visualization: PH. Writing – original draft: PH. Writing – review and editing: PH, AH, AHS.

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